VDAC Channels Mediate and Gate the Flow of ATP: Implications for the Regulation of Mitochondrial Function

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ABSTRACT The mitochondrial channel, VDAC, forms large (3 nm in diameter) aqueous pores through membranes. We measured ATP flow (using the luciferin/luciferase method) through these channels after reconstitution into planar phospholipid membranes. In the open state of VDAC, as many as 2×10^6 ATP molecules can flow through one channel per second. The half-maximum rate occurs at ~75 mM ATP. The permeability of a single channel for ATP is 1.1×10^{-14} cm^3/s (about 1 cm/s after correcting for cross-sectional area), which is 100 times less than the permeability for chloride and 10 times less than that for succinate. Channel closure results in a 50% reduction in conductance, showing that monovalent ions are still quite permeable, yet ATP flux is almost totally blocked. This is consistent with an electrostatic barrier that results in inversion of the selectivity of the channel and could be an example of how large channels selectively control the flow of charged metabolites. Thus VDAC is ideally suited to controlling the flow of ATP between the cytosol and the mitochondrial spaces.

INTRODUCTION

The flux of metabolites across the mitochondrial outer membrane is crucial to mitochondrial function and cell viability. The high permeability of the outer membrane is due to the presence of voltage-gated channels, called VDAC (or mitochondrial porin). VDAC has recently been shown to be responsible for most of this flux by measuring the permeability of the outer membrane in mitochondria from cells lacking VDAC genes (An-chin Lee, personal communication). The existence of large anion-selective channels in the outer membrane fits nicely with the major function of mitochondria, energy transduction. Substrates and products are mostly negatively charged molecules: succinate, pyruvate, ADP, ATP, phosphate, etc. There are a number of indirect indications that VDAC provides a pathway for nucleotide transport across the mitochondrial outer membrane. Experiments on intact mitochondria show that treatments that close VDAC greatly inhibit mitochondrial function and adenine nucleotide flux (Benz et al., 1988; Liu and Colombini, 1992a; Gellerich et al., 1993; Lee et al., 1994). Experiments on VDAC reconstituted into liposomes showed that these became leaky to many small molecules, including ATP (Báthori et al., 1993). However, the experiments reported here are the first to measure the ATP flux through VDAC. Early results were published as a rapid communication (Rostovtseva and Colombini, 1996).

All VDAC channels described to date have a remarkably conserved set of biophysical properties (Colombini, 1994; Colombini et al., 1996). When reconstituted into planar phospholipid membranes, VDAC channels are open most of the time at low voltages (~10 mV). Raising the potential to either positive or negative values moves VDAC to the low-conducting "closed" states. The gating process causes the diameter of the pore to decrease from ~2.4–3.0 nm to 1.7–1.9 nm (Colombini et al., 1987; Mannella and Guo, 1990). The channel's selectivity goes from moderately anion selective in the open state to moderately cation selective in the closed state (Colombini, 1980; Zhang and Colombini, 1990; Benz and Brdiczka, 1992). The gating behavior of VDAC channels can be influenced by various agents. The following either increase the voltage dependence or favor the closed state: synthetic polyanions such as König's polyanion and dextran sulfate (Colombini et al., 1987; Mangan and Colombini, 1987), oncotic pressure (Zimmerberg and Parsegian, 1986), and cellular constituents such as NADH (Zizi et al., 1994) and the VDAC modulator (Holden and Colombini, 1988, 1993). When added to intact mitochondria, the polyanions, VDAC modulator, and NADH reduce the ADP-stimulated respiration and inhibit mitochondrial intermembrane kinases, such as adenylate kinase and creatine kinase (Benz et al., 1988; Liu and Colombini, 1992a; Gellerich et al., 1993; Lee et al., 1994). These findings support the hypothesis that adenine nucleotides flow through VDAC and that VDAC gating might regulate the permeability of the outer membrane and thus control mitochondrial function.

This conclusion was tenuous because important evidence was still lacking: the direct demonstration that ATP could really pass through the VDAC channel. Furthermore, it was not clear that voltage gating observed when VDAC was reconstituted into phospholipid membranes is related to the physiological control of the flux of metabolites like ATP. Indeed, VDAC in the open state is large enough to be permeable to organic anions such as ATP, but closure results in a mere 50% reduction in channel conductance, and

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the estimated diameter of the "closed" channel is larger than the dimensions of metabolites such as ATP.

To obtain direct evidence, it was necessary to measure the ATP flux through the open and closed states of VDAC. For this purpose, the system of reconstituted VDAC channels into planar phospholipid membranes was used because it permits direct control of the state of the channels, the determination the number of channels responsible for the flux, and the specific measurement of the flux of ATP.

Here we report the results of direct measurements of ATP flux through VDAC channels reconstituted into the planar phospholipid membranes and held in either the open or closed state.

MATERIALS AND METHODS

Conductance measurements

VDAC channels were isolated, using standard methods, from *N. crassa* mitochondria (Mannella, 1982) and purified (Freitag et al., 1983).

Bilayer membranes were formed from monolayers made from a 1% solution of diphytanoylphosphatidyl choline (Avanti Polar Lipids, Alabaster, AL) with cholesterol (Sigma Chemical, St. Louis, MO) (10:3) in hexane following a modification of the Montal-Mueller technique (Montal and Mueller, 1972). The membrane potential was clamped by controlling the voltage on the *cis* side, while the *trans* side was maintained at ground by the amplifier. Ag/AgCl electrodes were used to interface with the solution via 1 M KCl-agar bridges. The transmembrane potential was clamped with an amplifier in the inverted mode (Schein et al., 1976), and the current across the membrane was recorded on a chart recorder. The membranes were made in asymmetrical solutions: 1 M KCl (*cis* side) and 0.1 M KCl (*trans* side), each containing 5 mM $MgCl_2$ and buffered with 10 mM HEPES (pH 7.0). Channel insertion was achieved by adding 1–3 μl of a 50× dilution of pure VDAC in 1% Triton X-100 into the aqueous phase on one side of the membrane (*cis* compartment). To facilitate the measurements of very small ATP fluxes, the aqueous compartments on each side of the membrane were only 200 μl.

In each experiment, after a sufficient number of channels had inserted, the solution in the *cis* compartment was replaced by a higher density ATP-containing solution with 0.1 M KCl and 10 mM HEPES (pH 7.0) (a 2× solution of ATP, obtained as the disodium salt (Sigma), was neutralized with NaOH before dilution with equal amounts of a 2× KCl solution). The conductance and selectivity of the multichannel membrane were monitored continuously throughout the experiments by applying square pulses of ±2 mV amplitude at a frequency of 0.5 Hz on top of a constant voltage offset (the desired applied voltage). For each experimental condition the applied voltage was chosen to hold the channels in either the open or closed conformation.

At the end of each experiment, the voltage-dependent properties of the VDAC-containing membrane in the presence of the ATP gradient were assessed by applying a symmetrical 5-mHz triangular voltage wave, ±60 mV in amplitude, and recording the current through the channels. The recordings were digitized and converted to conductance values. Only that part of the wave during which the channels were reopening was used for subsequent analyses.

To determine the number of open and closed channels at any time during the experiment, the single-channel properties were recorded under similar experimental conditions in parallel single-channel experiments. After insertion of the channel, a symmetrical 5-mHz triangular voltage wave ±60 mV amplitude was applied and the transmembrane current was simultaneously recorded. Then the 1.0 M KCl solution in the *cis* compartment was replaced by the ATP solution as described above. The conductances of single VDAC channels in the open and closed states were measured from the slopes of these recordings at the lowest possible potentials (rectification was clearly visible). Open- and closed-channel reversal potentials were measured by extrapolating the current tracings to $I = 0$. The number of open and closed channels was determined by dividing the value of the average conductance during the period of time between when the two samples (for ATP assay) were taken by the value of the appropriate single-channel conductance. The value of the single-channel reversal potential was used to verify that the channels were indeed in the appropriate conformational state.

ATP assay

The *trans* side was stirred continuously, and samples of 25 μl were taken periodically. The volume on the *trans* side was maintained by the addition of the same amount of fresh solution (0.1 M KCl, 5 mM $MgCl_2$, 10 mM HEPES, pH 7.0). The luciferin-luciferase (CLS and CLS II Kits; Boehringer-Mannheim, Germany) system was used to measure subnanomolar concentrations of ATP. The concentration of ATP in the samples (a volume ratio of reagent and sample of 1:1) was measured by comparing the light output to a calibration curve performed on the same occasion, using at least 12 different ATP concentrations (from 10^{-10} to 10^{-7} M). Calibration solutions were prepared by diluting, immediately before the measurements, an ATP stock solution with the same salt solution present in the *trans* compartment. A scintillation counter (1219 RACKBETA, LKB, WALLAC) out of coincidence was employed for the light measurements. The time to peak light emission was determined experimentally for each condition and varied from 2 to 10 min, depending on the kit used.

The ATP concentration in the stock and in the *cis* compartment was checked by measuring the absorbance at 259 nm and taking the extinction coefficient as 15.4×10^3. There was a substantial correction for water content in the ATP.

RESULTS

ATP flux through the open channel

By using the firefly luciferin-luciferase system to measure subnanomolar concentrations of ATP and a chamber with 200-μl compartments to minimize sample dilution, we have succeeded in measuring the flux of ATP through VDAC channels reconstituted into planar phospholipid membranes. The movement of 2.2 fmol ATP/min through a multichannel membrane is shown in Fig. 1. ATP flux remained constant during more than 5 h of experiment with channels in the open state. Under the experimental conditions used, these voltage-gated channels were open at low negative voltages (−20 mV in the experiment presented in Fig. 1).

To calculate the ATP flux per channel it was necessary to know the total number of channels during the experiment. By recording the single-channel properties under similar experimental conditions, the conductance and reversal potential values of VDAC were measured for the open and closed states. Fig. 2 illustrates a typical current recording from a single-channel membrane. A triangular voltage wave was applied to assess both conductance (slope) and reversal potential (zero-current intercept). The presence of an ATP gradient (80 mM ATP on the *cis* side) shifts the voltage at which gating is observed (as expected for any ion gradient; Zizi et al., manuscript in preparation). Thus the channel was open at low negative potentials (steep slope) but closed (entered a lower-conductance state) at positive and high negative potentials. It is evident that closure at negative potentials occurred over a rather narrow voltage region that

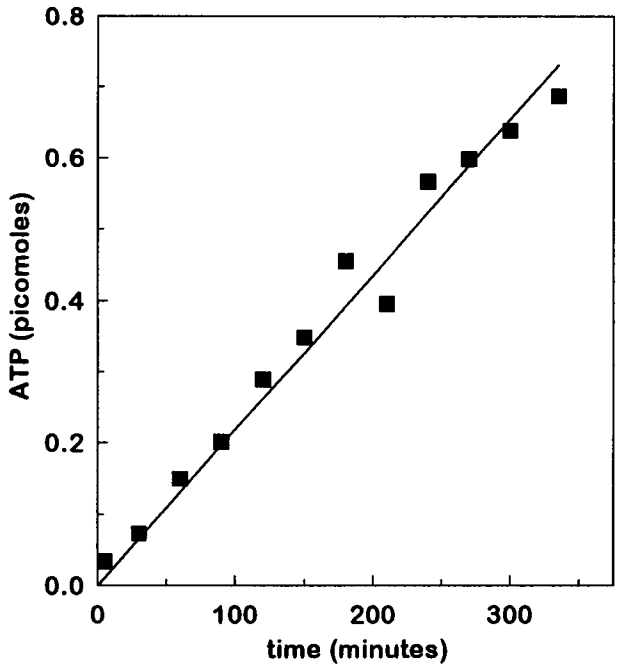

FIGURE 1 The ATP flux through VDAC channels in the open state. The figure shows the amount of ATP in the *trans* compartment as function of time. The *cis* ATP concentration was 35 mM. A voltage of −20 mV was applied during the experiment to hold the channels in the open state. The average number of channels was 39.

is accessible experimentally (extended application of higher negative potentials resulted in membrane instability).

Single-channel experiments were performed at various ATP concentrations so as to match the conditions used for the multichannel experiments. These data were used to calculate the number of open and closed channels from recordings of conductance and reversal potential monitored continuously throughout the flux experiments. To compensate for any changes in the number of channels in the membrane during the experiment, the ATP flux data (as in Fig. 1) were replotted versus the number of open channels times the time these were open before taking a sample. The ATP fluxes through the VDAC channel in open state were calculated with this approach (Fig. 3). The flux per single channel increased monotonically up to 2×10^6 ATP/s, where it saturated at an ATP concentration of about 150 mM.

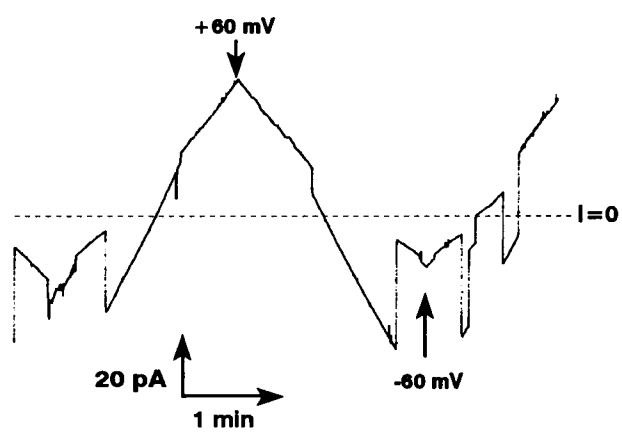

FIGURE 2 The voltage dependence of a single VDAC channel in the presence of an ATP gradient. The current trace was recorded with the voltage changing continuously (±60 mV) as described in Materials and Methods. The *cis* ATP concentration was 80 mM. A steep slope in the current trace indicates a high conductance and corresponds to the open state. Transitions to lines with shallower slopes are channel closures. The reverse is an opening. Time proceeds from left to right, and the zero-current level is indicated by the dotted line.

The voltage dependence of the conductance

The probability that a VDAC channel is open or closed at different potentials in the presence of an ATP gradient was estimated by obtaining *G/V* plots as shown in Fig. 4. Slow triangular voltage waves (5 mHz) were applied to multichannel membranes and the current was recorded. The data shown in Fig. 4 were collected by this method during a typical experiment in which ATP fluxes were measured. Because VDAC channels have rapid opening rates (submillisecond) and slow closing rates (seconds), a near-equilibrium *G/V* plot can be obtained by collecting data when the voltage changes in such a way as to open the channels. The solid symbols were collected as the voltage was increasing

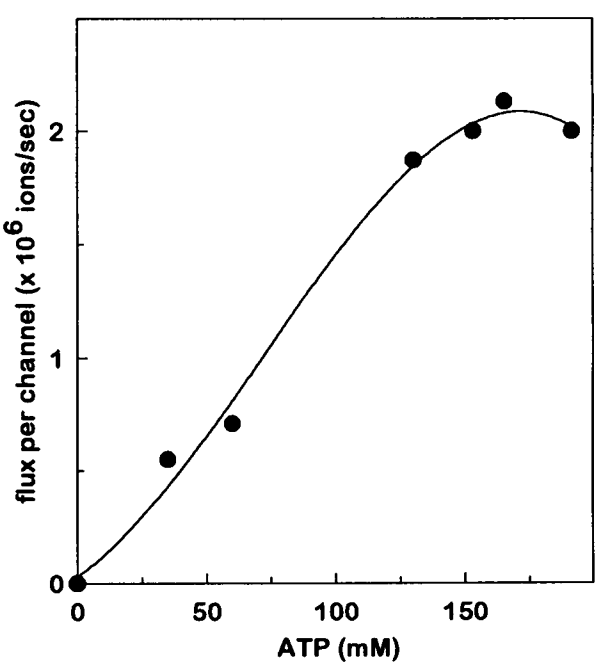

FIGURE 3 The dependence of the ATP flux through the VDAC channel on the ATP concentration in the *cis* compartment. The [ATP] in the *trans* compartment was essentially zero. Each point represents one experiment and is the value of the slope of the fitted line to the amount of ATP versus open channel-minutes. This was converted to ATP ions per channel per second.

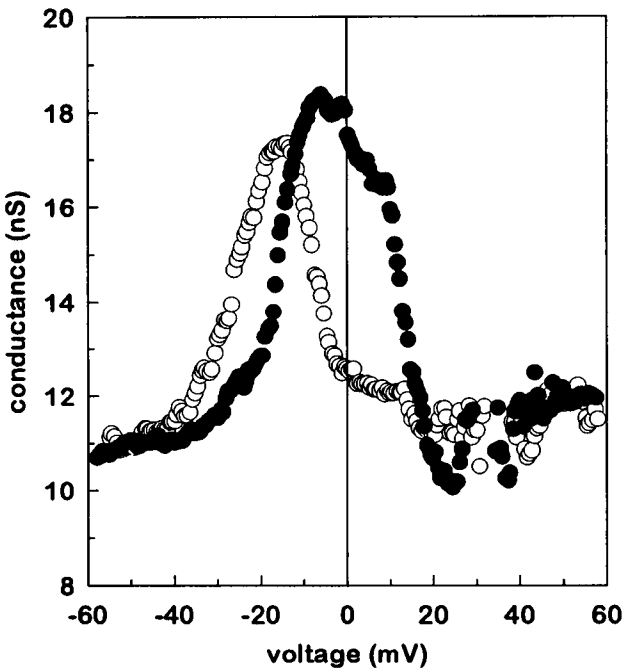

FIGURE 4 The voltage dependence of the conductance of VDAC channels in the presence of an ATP gradient. The *cis* ATP concentration was 130 mM. The data shown were obtained by analyzing the response of a multichannel membrane (35 channels) to a series of voltage ramps. The experimental conditions were as described in Materials and Methods. ●, Voltage change from negative to positive potential values; ○, voltage change from positive to negative values.

from −60 to +60 mV. The open symbols were collected as the voltage was declining. Thus the "equilibrium" *G/V* plot is the bell-shaped curve bounded on the right by open symbols and on the left by closed symbols. The channels are open over a very narrow region between −10 and −15 mV, making it difficult to find potential values at which the channels are totally open. However, by holding the membrane at a low negative voltage and giving transient pulses to −30, the channels could be kept open.

From this plot one might ask why the closed-state experiments at high negative voltages were not performed at −50 or −60 mV. It was a tradeoff between applying enough voltage to close the channels and keeping the voltage low enough to reduce membrane instability.

From Fig. 4 it is evident that the "closed" states at positive potentials and at high negative potentials are still quite conductive. There is a 10% difference in the conductance of the open state, depending on whether the voltage of the triangular wave was rising or falling. This is largely due to the rectification of the open channels in the presence of the sodium ATP gradient (see single-channel record, Fig. 2). The extent of rectification increased with increasing [ATP]. However, there may also be some hysteresis that may arise from the direction of the changing voltage.

Lack of ATP flow through the closed state

Closure of VDAC channels with positive potentials stopped ATP flow. As shown in Fig. 2, positive potentials cause channel closure, monitored as a conductance decrease and a reversal potential change from positive to negative values. Fig. 5 *A* shows the results of an experiment where the channels were held open for 100 min, closed for 100 min, and then reopened. The amount of ATP that crossed the membrane increased linearly while the channels were held open (at −10 mV). Raising the voltage to 25 mV closed the channels (low conducting state) and resulted in no significant flow of ATP. Upon channel reopening, the ATP flux resumed. The resumed flux was actually higher because of the presence of more channels in the membrane. These often insert spontaneously during the experiment. Replotting the data in Fig. 5 *A* against the product of number of open channels times the time they were open (Fig. 5 *B*) shows that the experimental points fit a straight line. This means that not only is there no significant ATP flux while the channels are held in the closed state, but also that all of the flux can be accounted for by the number of open channels and the length of time these were open.

The application of a positive voltage on the high-salt side not only closes the channels but also introduces an electric field unfavorable to the flow of ATP. Despite the presence of a nearly infinite ATP concentration gradient, this field is of concern. Therefore it was important to measure the ATP flux through the closed state induced at high negative voltages, where the electric field favors ATP flux. The difficulty is that it is hard to ensure that all channels are closed under these conditions (Fig. 4). Nevertheless, the result of such an experiment is presented in Fig. 6. Under a −10 mV applied potential, all 30 channels were open and ATP flux per a channel was 2.1×10^6 ATP/s. The application of −39 mV caused the channels to close, as monitored by changes in conductance and reversal potential. There was some ATP flux (0.37×10^6 ATP/s/channel) under these conditions, but it was 6 times less than that measured at −10 mV. The variability of the properties of the closed channels did not allow us to be certain that all channels were closed during the entire period at −39 mV.

The ATP flux under 0 mV applied voltage is shown in Fig. 7. Under these conditions, the channels were closed. It is obvious that in the absence of a transmembrane potential the ATP flux was essentially zero during 100 min of experiment.

ATP and the single-channel conductance

The presence of ATP caused an unusual increase in the conductance of the channels. In the presence of symmetrical 0.1 M KCl, the additional presence of ATP on the *cis* side increased the single-channel conductance much more than might be expected from the ATP and the associated Na^+ ions (Fig. 8). Certainly, from direct ATP flux measure-

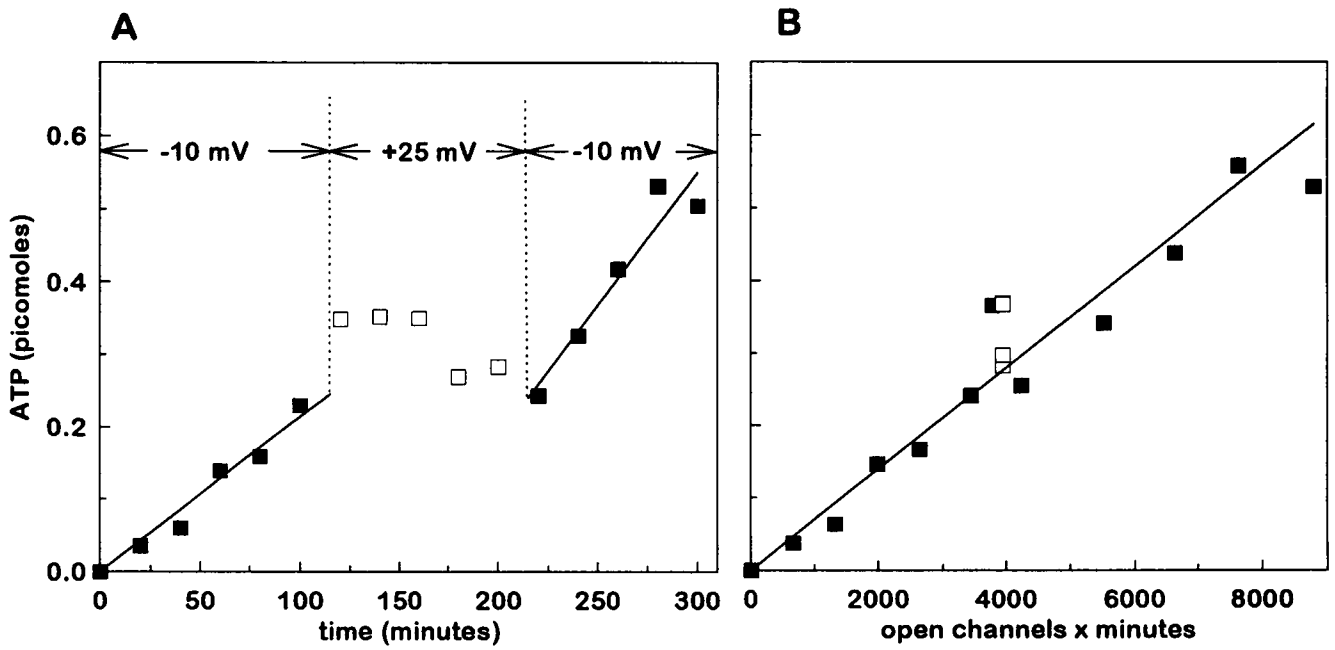

FIGURE 5 The ATP flux through the open and closed states of VDAC channels. The *cis* ATP concentration was 60 mM. The indicated voltage was applied to control the state of the channel. ■, Samples collected while the channels were open; □, samples collected when the channels were closed. (*A*) Amount of ATP in the *trans* compartment as function of time. (*B*) The data in *A* are replotted against the product of the number of open channels and the time these were open just before each sample was taken.

ments, the current contributed by ATP flux is minor. The biphasic nature of the curve is consistent with two ATP binding sites, one enhancing the conductance and the other diminishing it.

Permeability of the open state to ATP

Values for the permeability (P) of VDAC channels to ATP can be calculated from the measured fluxes by using the

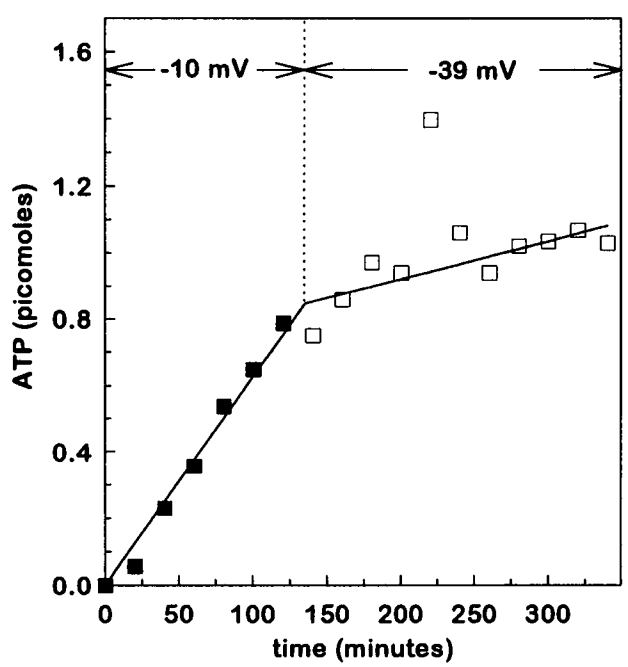

FIGURE 6 The ATP flux through VDAC channels before and after closure at elevated negative potentials. The *cis* ATP concentration was 165 mM. The voltage was applied to the *cis* side as indicated. ■, □, Open and closed states of the channel, respectively.

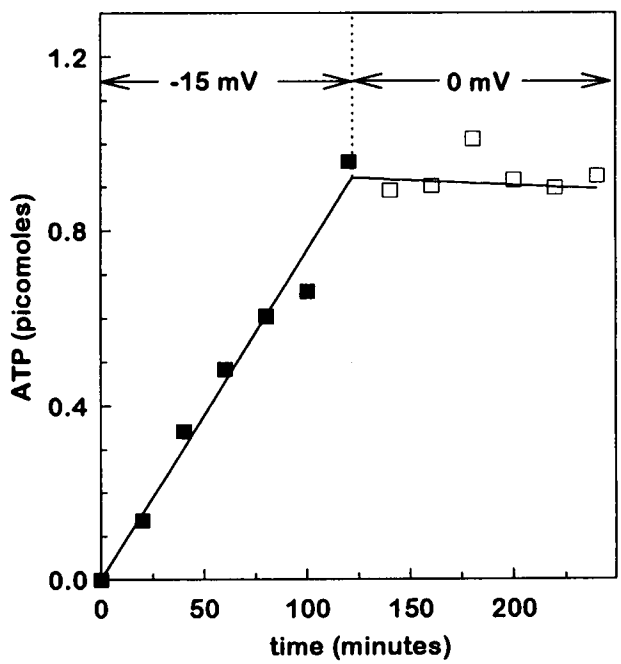

FIGURE 7 The ATP flux through VDAC channels before and after closure with zero potential. The *cis* ATP concentration was 153 mM. The applied voltage is indicated. ■, □, Open and closed states of the channel, respectively.

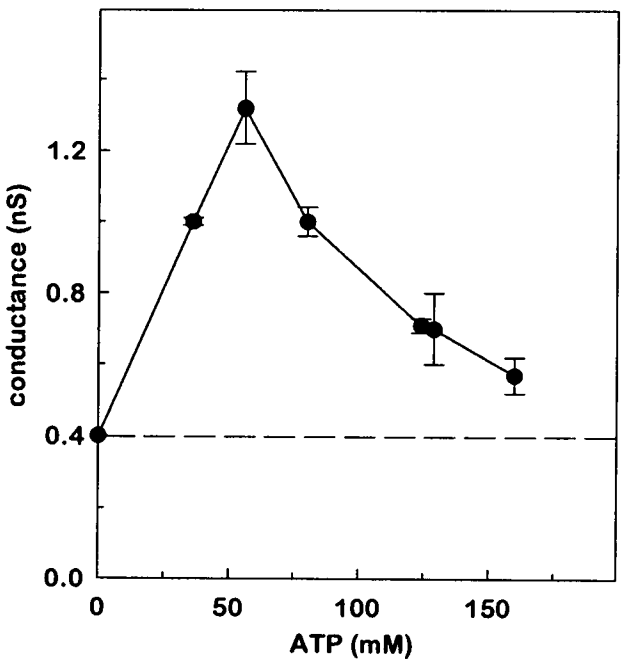

FIGURE 8 The conductance of a single VDAC channel in the open state at different ATP concentrations on the *cis* side. The single channel conductance was measured as a slope of an *I/V* plot, as illustrated in Fig. 2, when the channel was open. The slopes were taken at low electric fields. Each point is an average of at least 10 slopes obtained for the same channel. The absence of vertical bars means that the standard deviation is smaller then the size of the symbol.

Nernst-Planck flux equations to factor out the driving forces, the electric field, and the phenomenological force of the concentration gradient. By combining the flux equation with the definition for permeability, $p = D/dx$, one obtains

$$P = \frac{\text{Flux}}{\Delta C + (zFC/RT)\Delta\Psi},$$

where ΔC is the difference in [ATP] (just the [ATP] on the *cis* side), C is the ATP concentration inside the channel, $\Delta\Psi$ is the potential difference across the membrane, F is the Faraday constant, R is the gas constant, T is an absolute temperature, D is the diffusion constant, and z is the ATP valence, equal to -4 (at pH 7.0, 75% of ATP is in the form of ATP^{4-}). The P values were calculated assuming that C is half the ATP concentration on the *cis* side (Table 1). To obtain the permeability per effective channel area (P_0), the P values were divided by the effective pore size of the channel for ATP. The estimated pore radius, 1.2–1.5 nm, was reduced by the Stokes-Einstein radius of ATP (0.7 nm), and the resulting area is $0.79–2.0 \times 10^{-14}$ cm^2. Taking the average value for P from the table, the $P_0 = 0.56–1.4$ cm/s. If the channel length were 5 nm, this would be equivalent to a diffusion constant of $0.28–0.72 \times 10^{-6}$ cm^2/s. The diffusion coefficient for ATP in bulk water is 3×10^{-6} cm^2/s (Diehl et al., 1981), and thus the movement of ATP in the channel is 4 to 11 times slower than in bulk water.

TABLE 1 ATP fluxes and permeability through VDAC channel

ATP (mM)	V (mV)	Flux $\times 10^{-18}$ mol/s	Permeability $\times 10^{-14}$ cm^3/s
35	−20	0.92	1.01
60	−10	1.18	1.09
130	−10	3.12	1.33
130	0	1.75	1.35
153	−15	3.33	0.99
165	−10	3.55	1.20
191	−10	3.33	0.97
Average			1.13 ± 0.15

Permeability of the closed state to ATP

The apparent discrepancy between lack of ATP flux through the closed state at zero and positive potentials and the apparent measurable flux at elevated negative potentials can be understood in terms of the difference in electric field. If the ATP flux measured in Fig. 6 at −39 mV is through the closed state, the calculated permeability would be 9.1×10^{-16} cm^3/s. The corresponding flux at 0 mV would be expected to be 1.5×10^{-19} mol/s. This value is within the 90% confidence interval for the slope of the 0 mV data (Fig. 7), i.e., $0.2 \pm 3.7 \times 10^{-19}$ mol/s. Thus no discrepancy exists, and there may be significant permeability by ATP through the closed state under these conditions.

DISCUSSION

The experiments reported here show unambiguously that ATP can cross membranes through VDAC channels. The flux per single channel increased with increasing ATP concentration up to about 150 mM ATP, where saturation is evident (Fig. 3). Both the saturation of ATP flux with increasing ATP concentration and the biphasic nature of the single-channel conductance as [ATP] was elevated indicate some form of ATP binding to VDAC. The latter observation is best explained with two binding sites, one enhancing conductance and the other reducing conductance. Evidence for the presence of ATP-binding sites on VDAC has already been reported (Flörke et al., 1994). However, low levels of ATP (up to 5 mM) were not found to influence the gating properties of the channel (Zizi et al., 1994).

The estimated diffusion coefficient of ATP within the channel was found to be an order of magnitude lower than that in free solution. This is quite similar to that reported for the diffusion of uncharged polymers through the alamethicin channel (Bezrukov et al., 1994). We speculate, as they did, that this reduction in ability to diffuse may arise from friction with the walls and/or a higher viscosity of the water in the pore.

From the concentration dependence of the flux, one can roughly extrapolate down to physiological levels of ATP (1 mM) and calculate an estimated single-channel flux of 10^4

ATP/s. This is 100 times higher than the minimum ATP flux needed to account for the measured rates of ATP production in rat liver mitochondria. These rates, typical of mitochondria in general, are 11 nmol ATP/s/mg mitochondrial protein (Chappell, 1964). Good estimates of the amount of VDAC in these mitochondria have been published (Linden et al., 1984): 0.3% of the mitochondrial protein is VDAC. If all of the VDAC channels were open, the ATP flux per channel would be 70/s. Thus even though VDAC serves other functions (binding to kinases and microtubule-associated proteins, complexed to the benzodiazapine receptor, forming structures at contact sites) and only some of the channels may be open, there should be sufficient capacity to serve as the only conduit for ATP flux. If the permeability were substantially reduced, the outer membrane would become rate limiting, resulting in concentration gradients of ATP between the intermembrane space and the cytosol.

A comparison of the permeability of VDAC to ATP with its permeability to other anions is shown in Table 2. It is clear that there is a strong charge dependence on the permeability. Thus, although the electrostatic environment within the pore favors the translocation of anions, multivalent anions have reduced permeability.

Voltage-dependent channel closure is known (Colombini, 1980, 1989; Benz et al., 1990) to result in a reduction in conductance and inversion in selectivity. However, it is also known (Zhang and Colombini, 1990) that a family of closed states is accessible. Elevated voltages induce channels to enter states of even lower conductance. Application of voltages for long periods of time results in channels becoming adapted to the closed states and opening with difficulty and/or at slower rates. Thus the process is quite complex. The family of states is reduced when agents such as König's polyanion (Colombini et al., 1987) or the intermembrane protein, called the VDAC modulator (Liu and Colombini, 1992b), are used to favor closure. Nevertheless, these induce VDAC entry into states that are almost totally impermeable to ions. Despite this complexity, the readily accessible closed states accessed by the conditions used in the experiments reported here are clearly closed to ATP. The closed states that have an even lower conductance may be designed to restrict the flow of other substrates.

TABLE 2 Comparison of VDAC permeability for various anions

Anion	Flux at $V = 0$* ($\times 10^6$) ions/s	Permeability ($\times 10^{-14}$) cm^3/s
ATP^{-4}	1.1	1.1#
Cl^-	67	110
HPO_4^{-2}	6.3	10
$Succinate^{-2}$	6.8	11
$Citrate^{-3}$	3.4	5.6

*Data for anions other than ATP are from Hodge and Colombini (1997) and were collected in the presence of 200 mM salt versus 100 mM salt. Datum for ATP is from Table 1, 0 mV experiment.
#Average permeability from Table 1.

A change in a pore diameter from 2.5–3 nm in the open state to 1.7–1.8 nm in the close state cannot, by itself, account for this dramatic change in ATP permeability. If the closed state allows the easy passage of the tetrose, stachyose, it should show little steric resistance to ATP. However, the channel goes from moderately anion selective (5:1, Cl^- over K^+) in the open state to moderately cation selective (6:1 K^+ over Cl^-) in the closed state (Hodge and Colombini, 1997). As illustrated in Fig. 9, a number of experiments point to the conclusion that a portion of the VDAC channel moves upon channel closure out of the lipid bilayer (Blachly-Dyson et al., 1990; Peng et al., 1992; Thomas et al., 1993; Colombini, 1994; Colombini et al., 1996). Because the net charge on this part is positive, this motion would account for the reduction in pore volume and diameter and the change in selectivity because the remaining transmembrane strands would result in a net negative charge in the pore. It was estimated (Colombini, 1980; Zambrowicz and Colombini, 1993) that the net charge of the wall of the pore goes from a net positive charge of 3 to a net negative charge of −3. This generates an electrostatic barrier that is probably responsible for excluding ATP (Fig. 9). High

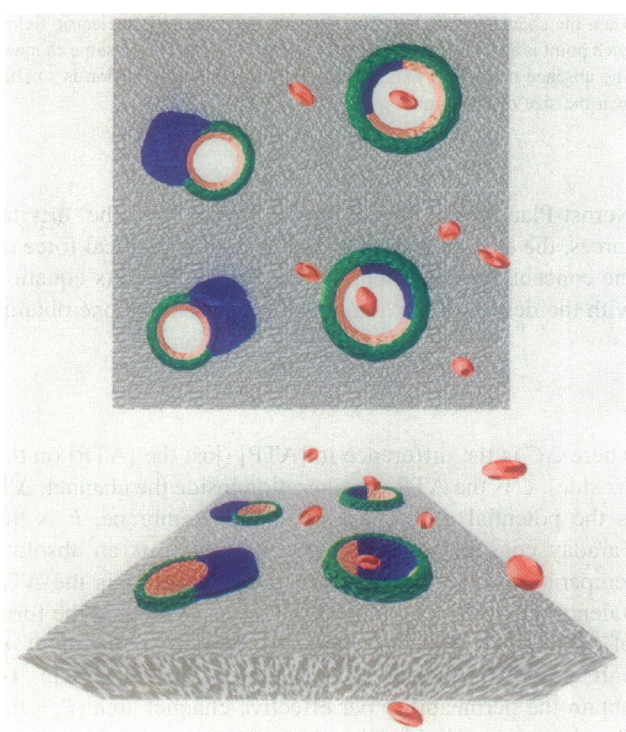

FIGURE 9 A working model of VDAC regulation of ATP flux. A rectangular section of membrane containing four channels is illustrated both in oblique view (*lower*) and top view (*upper*, as if reflected by a mirror). The design of the channels embodies our current understanding of the structure of VDAC and of its gating process. The membrane potential drives a portion of the wall of the channel, which serves as a positively charged voltage sensor (*blue*), out of the channel during the closing process, resulting in a net negatively charged region (*pink*) within the pore. The swarm of ATP molecules is illustrated as red ellipsoids. The relative sizes of the pores and ATP are about to scale.

electric fields could still drive ions, especially tetravalent ions, through this barrier. This could account for the measured flow at high negative potentials. This is an example of how a large channel can selectively control the flow of charged metabolites. Another indication of the electrostatic barrier overwhelming steric considerations comes from observations on the cardiac ryanodine receptor channel (Tu et al., 1994). There, a reduction in conductance resulted in *less* single-file behavior, indicating a larger pore.

VDAC channels are the primary pathway for the flow of metabolites across the mitochondrial outer membrane. Their highly conserved properties include a variety of regulatory mechanisms that could restrict the flow of metabolites between the cytoplasm and the mitochondrion. Regulating proteins and NADH have been shown to be capable of regulating the opening and closure of VDAC (Holden and Colombini, 1988; Brdiczka et al., 1994; Zizi et al., 1994). Voltage across the outer membrane can easily be generated by differences in charged impermeable macromolecules (Donnan potential) between the two compartments or asymmetrical surface potentials. This could be altered by means of protein phosphorylation or Ca^{2+} binding and thus could be responsive to established cellular regulation pathways. Mitochondrial activity was shown to be dramatically affected by the release of Ca^{2+} from cellular stores (Sparagna et al., 1995; Fein and Tsacopoulos, 1988). This change may occur at the crest of every intracellular Ca^{2+} wave as Ca^{2+} is accumulated into and released from the matrix space (Hajnoczky et al., 1995).

Skeptics voice the opinion that VDAC is unlikely to function as a regulator of mitochondrial function because it forms a large conductive pore in both the open and closed states. However, the high level of molecular traffic between the cytosol and mitochondrial compartments that must occur in vivo may require large channels to be open. In addition, the relatively large size and often large charge on these metabolites should contribute to the selection pressure that maintains these highly conserved characteristics in VDAC. Yet regulation is clearly possible, because VDAC closure results in a drastic drop in ATP flux while simultaneously allowing small, monovalent ions to flow through the membrane. Thus regulation may be a key part of VDAC function, as it is for most cellular processes.

These results clearly demonstrate that VDAC channels in the mitochondrial outer membrane can not only facilitate but regulate the movement of ATP between the cytosol and the mitochondrial spaces. Thus VDAC channels are ideally suited to controlling ATP flux through the mitochondrial outer membrane, and consequently mitochondrial function.

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